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Evaluation of the Protective Effect of Ethanolic Extract of *Plantago major* Plant Against CCl4 Induced Genotoxicity and Biochemical Disturbance in Rat

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THIS STUDY was carried out through 2010-2014, seasons on fresh herbs of *Plantago major* plant, which was collected from a private nurseries of El Qanater el Khayreyya, Al- Qaluobia Governorate, Egypt. The aim of this study was to investigate the protective potential of ethanolic crud extract of *Plantago major* against oxidative stress induced by carbon tetrachloride (CCl_4) in different tissues in rats. Our results observed that CCl4 exhibited a significant increase in the levels of serum glucose and lipid profile *i.e.* total cholesterol, triglyceride and low density lipoprotein cholesterol (LDL) with a significant decrease in the level of high density lipoprotein cholesterol (HDL). Also, CCl4 increase liver function including serum aminotransferases (AST and ALT), alkaline phosphatase (ALPh), total protein, albumin (ALB) and total bilirubin activities and kidney function including the levels of serum urea, uric acid and creatinine amounts. Treatment with ethanolic *P. major* extract at 100mg/kg b.wt before, with and after treatment with CCl4 significantly prevented all of these typically observed changes . Also, *P. major* extract statistically significant (P<0.01) inhibit DNA damage induced by CCl4 in bone marrow and sperms of rats. Our findings indicate that *P. major* has a significant protective effect against CCl4 induced genotoxicity and biochemical changes in rat, which may be due to its antioxidant properties.

Keywords: *Plantago major* herb, Carbon tetrachloride, Chromosomal abnormality, Biochemical analysis.

Carbon tetrachloride (CCl_4), a clear, colorless, volatile, heavy and nonflammable industrial liquid, widely used to induced free radical toxicity in various tissues of experimental animals such as liver, kidneys, heart, lung, testis, brain and blood (Khan *et al.,* 2010). CCl_4 is converted through hepatic microsomal cytochrome

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P450 into trichloromethyl-free radical (CCl_3 or CCl_3OO) (Preethi and Kuttan, 2009) which in turn, initiate lipid peroxidation process (Adewole *et al.,* 2012). The most widely accepted mechanism of CCl_4 induced hepatotoxicity is the formation of free radicals which is a rate limiting process in tissue peroxidative damage (Sahreen *et al.,* 2011 and Khan *et al.,* 2011). This free radical and related reactive species may cause oxidative stress, which produces major interconnected changes of cellular metabolism, increases the serum marker enzymes, DNA fragmentation, and destruction of the cells by lipid peroxidation (Bhadauria *et al.,* 2008). The accumulation of lipid peroxides introduces hydrophophilic moieties and alters membrane permeability and cell function which causes the loss of hepatic integrity and depressed hepatic function resulting in hepatotoxicity and congestive hepatic failure (Khan *et al.,* 2012). To protect the body from such deleterious effects of free radicals, several endogenous enzymatic and non enzymatic systems are provided, but when the formation of free radicals is excessive, additional protective mechanisms of dietary antioxidants may be of a great importance (Tirkey *et al.,* 2005). Maintaining the balance between reactive oxygen species and natural antioxidants is therefore crucial, and could serve as a major mechanism in preventing damage by oxidative stress induced by toxic agents. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes (Sreelatha *et al.,* 2009). Antioxidant and radical scavengers have been used to study the mechanism of CCl_4 toxicity as well as to protect tissue cells from CCl_4 induced damage by breaking the chain of lipid peroxidation (Weber *et al.,* 2003). Numerous studies have shown that horticultural crops and fruits are sources of diverse antioxidant properties, which can protect body against CCl_4, induced oxidative stress (Ogeturk *et al.,* 2005).

With respect to cytotoxic, genotoxic or mutagenic activity of chlorinated aliphatic hydrocarbons the data showed that the DNA reactivity such of compounds increased with increasing degree of halogenisation (Tafazoli and Kirsch-Volders, 1996). The carcinogenic potential of carbon tetrachloride has been early shown by (Weisburger, 1977) in mice in which liver and adrenal tumors were induced, and in rats in which disseminated neoplastic nodules were observed after oral exposure. The positive clastogenic effect has been demonstrated by *in vitro* conditions in metabolic competent.MCL-5 and h2E1 human cell lines (Doherty *et al.,* 1996) and in workers occupationally exposed to mixture of chlorinated solvents or carbon tetrachloride in comparison to the group that was not exposed (Da Silva *et al.,* 1997). Dianovsk and Ivikova (2001) showed a significant increase of sister chromatid exchange (SCE) in sheep peripheral lymphocytes after CCl4 treatment. Tafazoli *et al.* (1998) pointed out in their report that mutagenicity of CCl4 occurred in absence of significant cytotoxicity.

Medicinal and aromatic plants use by 80% of global population for their medicinal therapeutic effects as reported by WHO (2008). Many of these plants

synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Others contain alkaloids, glycosides, saponins, and many secondary metabolites (Naguib, 2011).

Among many popular medicinal plants, plantago has accessed a scientific value, as it has been taken place in many historical uses as a wound healing remedy for centuries (Dokuparthi and Manikanta, 2015). *P. major* is commonly called as "blonde psyllium" in English and "Isabgol" in Hindi and Urdu, *P. major* is the member of the family of Plantaginaceae, which is a vastly diverse genus, including around 256 species (Dhar *et al.,* 2005).

P. major contained active components such as, mucilages, flavonoids, phenolic acids, hydroxycoumarins, tannins and antioxidant activities (Blumenthal *et al.,* 2000). Phytochemical studies have also shown that the genus *Plantago* contains a great amount of phenolic compounds (flavonoids and tannins). In particular, phenolic compounds seem to play a potential role in the control of bacterial growth and could prevent tooth decay, decreasing growth, and virulence of pathogenic oral flora (Smullen *et al.,* 2007). A recent study has reported that *P. major* methanol/water extracts are rich in phenolic acids, with the benzoic acid derivatives hydroxybenzoate and 3,4,5-trihydroxybenzoate (gallic acid) being the most highly represented (Beara *et al.,* 2012).

Furthermore, experimental studies have proven that this plant has anti-ulcerogenic activities (Atta *et al.,* 2005), anti-inflammatory activities (Beara *et al.,* 2010), antigiardiasic activity (Ponce-Macotela *et al.,* 1994), antimalarial activity (Weenen *et al.,* 1990), anticancer activities (Mohamed *et al.,* 2011), immunomodulatory activity (Chiang *et al.,* 2003), wound healing activities (Zubair *et al.,* 2012), antioxidant activities (Stanisavljevic *et al.,* 2008 and Beara *et al.,* 2009), antimicrobial activities (Stanisavljevic *et al.,* 2008), antihypoglycaemic activity (Díez-Láiz *et al.,* 2015), decreases in the levels of serum BUN urea and serum creatinine (Hong *et al.,* 2011), hepatoprotective activity (Hussan *et al.,* 2015), hyperlipidemic activity (Hu *et al.,* 2014) and haemopoietic and cytotoxic activity (Velasco-Lezama *et al.,* 2006).

The aim of the present study was to investigate protective effect of *P. major* ethanolic extract against CCl4-induced hepatotoxicity and genotoxicity in rats.

Material and Methods

Plant materials

Fresh herbs of *P. major* was collected form a private nurseries of El-Qanater El-Khayreyya, Al-Qaluobia Governorate, Egypt. Plant material was authentified by the Flora Department of Horticulture Research Institute, Agricultural Research Center, Giza governorate, Egypt (August 2010).

Chemical composition

Moisture, ash, crude protein, crude fiber and vitamin C.

Moisture, ash, crude protein, crude fiber and vitamin C were determined as recommended by A.O.A.C. (2005).

Minerals determination.

Nitrogen was determined by the modified microkjeldahl method as described by Pregl (1945). Phosphorus content was colorimetrically determined according to the method of Jackson (1958). Potassium was determined using Flame Photometer apparatus according to Jackson (1967).

Determination of total free amino acids.

Total free amino acids were determined by Jayaraman (1985).

Total sugars (Reducing – Non-reducing).

Total sugars were determined by Miller (1959).

Total carbohydrates.

Total carbohydrates were determined by Smith *et al.* (1956).

Determination of chlorophyll a &b and carotoinds.

Chlorophyll-A, chlorophyll-B and carotenoids were extracted from leaves according to the methods of Sweeney and Martin (1958).

Determination of total indoles compounds.

Extraction of indolic compounds was conducted according to the method described by Daniel and George (1972).

Fatty acid.

Fatty acid was determined as recommended by A.O.A.C. (2005).

Volatile oil (Essential oil).

Essential oils was determined according to the method of Guenther (1960).

Identification and determination of fatty acids.

Fatty acid profiles were determined according to the method of (Aura *et al.*, 1995) utilizing a gas chromatograph.

Preparation of ethanolic plant extract (Soxhlet extraction)

Plant materials were dried at oven temperature on 50°C, and grounded in a mortar. Two hundred and fifty grams of each plant powder was extracted in 1000 ml of solvent in dark colored bottles for the extraction process by maceration (36 h). Resulted suspension was filtered through white Whatman filter paper. Pellet was re-extracted using solvent repeatedly for 2 more times. But each time the amount of the solvent was decreased. Three or more times filtrates were collected to remove the used solvent using rotary evaporator at 40^{o}C. Crude extract was kept at -8^{o}C until further experiments.

Extract analysis for active ingredient

Total Phenols

Extraction of phenolic compounds was conducted according to the method described by Daniel and George (1972).

Total flavonoids

Total flavonoids were estimated using method of Sakanaka *et al.* (2005).

Identification of phenols by HPLC.

Phenols were fractionated using HPLC (HP 1100) according to Merfort *et al.* (1997).

Total antioxidant activity

The antioxidant activity of the extracts was studied through the evaluation of the free radical scavenging effect on the 2,2 diphenyle 1-1picrylhydrazl (DPPH). The determination were assessed by Brand-Williams *et al.* (1995).

Total alkaloids

Total alkaloids content was determined by Shamsa, et al. (2008).

Hydrolysable tannins content.

Hydrolysable tannins content were determined by the method of Çam and Hişil (2010).

Saponine content

Saponine content was determined as described by and Uematsu et al. (2000).

Detection for Terpenoid

A reddish brown precipitate coloration at the interface formed, indicated the presence of terpenoids according to Ganatra *et al.* (2012).

Experimental animals

Laboratory-bred strain male rat weighting of 200-250 g obtained from the animal house of Research Institute of Ophthalmology, Giza, Egypt. Animals were housed in groups (5 animals/group) and maintained under standard condition of temperature, humidity and light. The animals were given standard food and water *ad libitum*.

Experimental Design

For hepatoprotective evaluation of ethanolic extract of *P. major* against liver damage induced by CCl4 for 7 days.

The CCl4 model described by Shenoy *et al.* (2001) was used for scheduling the dose regimen. Intraperitoneal injection of 0.8 ml/kg CCl4 diluted in distilled water (3: 10 dilution) was employed for inducing acute liver toxicity.

Rats were divided into groups (5 animals/group) as follow

- Control group (non treated, negative control).
- CCl4 model group 0.8 ml/kg/day for 7 days (positive control).
- Ethanolic extract of *P. major* group with the dose 100mg/kg body weight for 7 days.
- Ethanolic extract of *P. major* group with the dose 100mg/kg body weight 4h before CCl4 treatment for 7 days.
- Ethanolic extract of *P. major* group with the dose 100mg/kg body weight simultaneously with CCl4 treatment for 7 days.
- Ethanolic extract of *P. major* group with the dose 100mg/kg body weight 4h after CCl4 treatment for 7 days.

Blood and liver samples were collected 24h after the last treatment. At the end of experiment, blood was collected in tubes from retro-orbital vein in separated tubes. The tube was centrifuged at 3000 rpm for 20 min, for serum preparation.

Biochemical assay

Serum glucose

Glucose presented in the sample was determined according to the procedure of Trinder (1969).

Lipid profile

- Determination of total cholesterol
- Total cholesterol was calorimetrically determined at 546 nm according to the enzymatic method described by Allain *et al*. (1974).
- Determination of triglyceride
- Serum triglyceride was determined according to the method described by Fossati and Principe (1982).
- Determination of high density lipoprotein cholesterol (HDL)
- Serum high density lipoprotein cholesterol was determined by the method described by Tietz *(*1976a).
- Determination of low density lipoprotein cholesterol (LDL)
- Serum LDL-cholesterol was calculated according to Tietz (1976a).

Liver functions

- Determination of serum aspartate transferase activities (AST-GOT)
- Serum aspartate transferase (AST) activities were measured calorimetrically according to the method of Tietz (1976b).
- Determination of serum alanine transferase activities (ALT-GPT)
- Serum alanine transferase (ALT) activities were measured colorimetrically according to the method of Tietz (1976b).
- Determination of alkaline phosphatase (AL Ph)
- Alkaline phosphatases (AL Ph) in serum were determined according to the method of Young *et al.* (1972).
- Determination of serum total protein
- Serum total protein was measured according to the method described by Vassault *et al.* (1986).

- Determination of Albumin (ALB)
- Albumin in serum was determined according to the method of Young *et al.* (1975).
- Determination of total bilirubin
- Bilirubin in serum was determined according to the method of Walters and Gerarde (1970).
- *Kidney function*
- Determination of serum urea
- Serum urea was determined according to Tietz (1990)
- Determination of serum creatinine
- Creatinine in serum was determined according to the method of Tietz (1986).
- Determination of uric acid
- Uric acid in serum was determined according to the method of Vassault *et al.* (1986).

Cytogenetic studies

Doses

For cytogenetic analysis, animals treated with the same doses and time of treatment as in hepatoprotective study. For somatic cells animals treated for 7days and samples collected 24h after the last treatment. For somatic cells preparation, animals from the different groups were injected i.p. with colchicine, 2-3h before sacrifice. In germ cells animals treated for 5days. Samples taken after 35 days from the first treatment (cell cycle of sperm preparation from spermatocyt cells takes about 35 days).

Chromosome aberrations in somatic cells

Chromosome preparations from bone - marrow (somatic cells) carried out according to the method of Yosida and Amano (1965). 100 well spread metaphases were analyzed per rat. Metaphases with gaps, chromosome or chromatid breakage, fragments, deletions, as well as numerical aberrations (polyploidy) were recorded.

Sperm-shape abnormalities

Sperm were prepared according to the recommended method of Wyrobek and Bruce (1978).1000 sperm were analyzed per rat. Sperms with straight, banana shape, amorphous, without hook, as well as coiled tail were recorded.

Evaluation of the activity of plant extract to reduce abnormalities induced by CCl4 was carried out according to Madrigal-Bujaidar *et al.* (1998) formula as follows

Inhibitory index (II) = [1- (plant extract and CCl4 – control) / (CCl4 - control)] X100

Statistical analysis

The complete randomized blocks design was adopted for the experiment. The statistical analysis of the present data was carried out according to the methods described by Snedecor and Cochran (1980). Averages were compared using the new LSD method at 5% level. For cytogenetic analysis the significance of the results from the negative control data and between plant extract with CCl4 comparing to CCl4 alone was calculated using t-test.

Results and Discussions

Chemical composition of P. major herbs

As shown in Table 1, data revealed that chemical composition of *P. major* herbs contain 95.56% moisture, 13.88% ash, 0.507% crude fiber, 8.061mg/100g F.W. vitamin c, 1.544% nitrogen, 0.458% phosphorus, 2.112% potassium, 1.173% total free amino acids, 9.653% crude protein, 4.038% reducing sugars, 1.497% non-reducing sugars, 5.534% total sugars, 3.499% total carbohydrates, 0.136mg/100g F.W. chlorophyll-a, 0.076mg/100g F.W. chlorophyll-b, 0.016mg/100g F.W. carotenoids, 8.277mg/100g F.W. total indoles, 2.099% total fatty acid and 0.13% essential oil. These results are harmony with those (Shad *et al.,* 2013, El-Sheikh 2014 and Ferrazzano *et al.,* 2015).

TABLE 1. Chemical composition of *P. major* herbs.

Moisture (%)	**95.56**
Ash (%)	13.88
Crude fiber (%)	0.507
Vitamin C (mg/100g F.W.)	8.061
Nitrogen (%)	1.544
Phosphorus (%)	0.458
Potassium (%)	2.112
Total free amino acids (%)	1.173
Crude protein (%)	9.653
Reducing sugars (%)	4.038
Non-reducing sugars (%)	1.497
Total sugars (%)	5.534
Total carbohydrates (%)	3.499
Chlorophyll-A (mg/g F.W.)	0.136
Chlorophyll-B (mg/g F.W.)	0.076
Carotenoids (mg/g F.W.)	0.016
Total indoles (mg/100g D.W.)	8.277
Total fatty acid (%)	2.099
Essential oil (%)	0.13

Identification and determination of fatty acids

Data in Table 2 showed that the total fatty acids of *P. major* contain 73.05% of the total unsaturated fatty acid, while the total saturated fatty acid were

26.95%. Lenoleic acid (C18:2) is the major component of the main unsaturated fatty acid, while the main saturated fatty acid is palmetic acid (C16:0).

The main components of fatty acids of *P. major* herbs was 27.96% for lenoleic acid (C18:2), followed in a descending order by 23.20% for linolenic acid (C18:3n3) followed by 15.56% for oleic acid (C18:1) followed by 15.44% for palmitic acid (C16:0) followed by 4.43% for myristoleic acid (C14:1) followed by 4.28% for lauric acid (C12:0) followed by 3.11% for myristic acid (C14:0) followed by 2.62% for stearic acid (C18:0) and less than 1% for palmitoleic acid (C16:1), margaric acid (C17:0), margaolic acid (C17:1), α - linolenic acid(C18:3n6), arachidic acid (C20:0), gadoleic acid (C20:1) and bahenic (C22:0).

The obtained results are in agreement with those reported by Liu *et al.* (2002) who detected that the total fatty acid content in *P. major* leaves is 286 mg per 100 g of fresh plant material, the major fatty acids were linolenic, linoleic, and palmitic acid, but smaller amounts of stearic, oleic, and myristic acid. Also, Javed *et al.* (2003) found that the *P. ovata* seed contain lipid percentage is rather low (5.2%) as compared to other oil bearing seeds. The total lipids were classified into neutral and polar lipids by thin layer chromatography. The neutral lipids identified, were hydrocarbons (1.3%) wax esters (2.1%), triacylglycerols (74.2%), free fatty acids (2.5%), 1,3-diacylglycerols (3.6%), 1,2-diacglycerols (3.9%), glycolipids (1.2%), fatty alcohols (2.0%) sterols (1.3%), 2-monoacylglycerols (2.4%) and 1-monoacylglycerols (3.0%). The fatty acid range was C 14.0 to C 18.3. All the lipids classes showed higher percentages of unsaturated fatty acids in which linoleic acid (C 18.2) is predominant one (45.9-61.9%). The other fatty acids were myristic (C 14.0) palmitic (C 16.0) stearic (C 18.0) Oleic (C 18.1) and linolenic acid (C 18.3), palmitic acid was the highest (8.5 - 13.9%) while myristic acid was the least (0.3 - 1.2%) in saturated acids profile. In addition to, Romero-Baranzini *et al.* (2006) mentioned that, *P. ovata* seeds contain 6.7% crude fats. In addition to, the oil from *Plantago* seeds had a high percentage of linoleic acid (40.6%) and oleic acid (39.1%) and a minor proportion of linolenic acid (6.9%). Moreover, Dungait *et al.* (2008) studied the gas chromatogram of fatty acids extracted from *P. media* leaves. They found a high abundance of saturated C16 and unsaturated C18 fatty acids. Average percentage fatty acid contents for C16:0, C18:2 and C18:3 of leaves analyzed were 20%, 13% and 56%, respectively.

TABLE 2. Chemical constituents of fatty acids for *P. major* herbs.

Chemical constituents of fatty acids	(%)
Lauric acid (C12:0)	4.28
Myristic acid (C14:0)	3.11
Myristoleic acid (C14:1)	4.43
Palmitic acid (C16:0)	15.44
Palmitoleic acid (C16:1)	0.68
Margaric acid (C17:0)	0.18
Margaolic acid (C17:1)	0.10
Stearic acid (C18:0)	2.62
Oleic acid (C18:1)	15.56
Linoleic acid (C18:2)	27.96
α -linolenic acid(C18:3n6)	0.72
Linolenic acid (C18:3n3)	23.20
Arachidic acid (C20:0)	0.62
Gadoleic acid (C20:1)	0.40
Bahenic (C22:0)	0.70
Un-saturated Fatty acid	73.05
Saturated Fatty acid	26.95

Active ingredient from ethanolic extract of P. major herbs

Data presented in Table 3 noticed that active ingredient activity of *P. major* herbs contain 31.67mg/100g D.W. total phenols, 90.14mg/100g D.W. total flavonoids, 951.9mg/100g D.W. antioxidants activity, 143.3 mg/100g D.W. total alkaloids, 15.23 mg/100g D.W. total tannins, 15.68 mg/100g D.W. total saponine and exhibited the presence of total terpenoid.

These results are in line with those (Salihoglu *et al.,* 2013, Reslon *et al.,* 2014, Mello *et al.,* 2015 and Abd El-Gawad *et al.,* 2015).

TABLE 3. Active ingredient from ethanolic extract of *P .major* herbs.

Active ingredient from ethanolic extract of *P. major*	mg/100g dry extract
Total phenols	31.67 Gallic acid
Total flavonoids	90.14 Quercetin
Antioxidants activity	951.9 DPPH
Total alkaloids	143.3 Atropine
Total tannins	15.23 Gallotannic acid
Total saponine	15.68 Diosganin
Total terpenoid	Test (+)

Identification and determination of phenols

Data presented in Table 4 performed to identify and determine the major constituents of phenols by ethanolic extract of *P. major*, it is found that ethanolic extract of *P. major* comprised the biologically active phenolic compounds including syringic, protocatechol, chatechcol, euganal, gallic, catechin, salicylic, ellagic, cinnamic and chrysin.

The obtained results are in agreement with those reported by Khalil *et al.* (2007) found that *P. afra* contain phenolic compounds including sallicylic acid, pyrogallic acid, coumaric acid, ferulic acid, resorcinol, phenol, coumarin, vanillin, chlorogonic acid, cinnamic acid, hydroxyl benzoic and catechol. Similarly, Beara *et al.* (2009) found that found that *Plantago* species methanolic extract including *P. argentea Chaix, P. holosteum Scop,. P. major* L., *P. maritima* L., and *P. media* L. contain flavonoids profile including luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin, rutin, and quercetin was identified. Also, Mohamed *et al.* (2011) found that *P. major* comprised the biologically active phenolic compounds including ferulic, caffeic, gallic acid and vanillic acids, in addition to flavonoid compounds, i.e., luteolin, kaempferol, rutin, naringenin and ferulic acid. In this respect, Jankovic *et al.* (2012) found that extract with 50% ethanol of the aerial parts of six *Plantago* contain flavonoid compounds fractions with different concentrations according to plantago species including luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin, apigenin, rutin, mangiferin. In addition to, Amakura *et al.* (2012) found that seven phenolic compounds, among them, vanillic acid, plantamajoside, desrhamnosyl acteoside, and new phenylethanoid glycoside, were isolated from the ethyl acetate extract of *Plantago* herb (whole part of *P. asiatica* L.).

TABLE 4. Chemical constituents of phenols compounds for *P. major* .

Chemical constituents of phenols compounds	(%)
Syringic	0.1
Protocatechol	23.7
Unknown	5.5
Chatechcol	6.7
Euganal	20.4
Gallic	9.5
Catechin	12.9
Salicylic	2.2
Ellagic	10.8
Cinnamic	1.3
Unknown	4.0
Chrysin	3.0

Biochemical assay

Effect of P. major plant extraction on rats injected with CCl4

As shown in Tables 5, 6 and 7, data revealed that the assessment of kidney (renal) and liver function impairment effect of carbon tetrachloride (CCl4) and the protective potential of *P. major* extract. Serum urea, uric acid and creatinine concentrations were studied to assess the renal functions while serum ALT, AST ALPh, total protein, ALB and total bilirubin levels were determined to evaluate the hepatic functions. CCl4 induced significant elevations in these parameters when compared to control values.

Serum glucose

Regarding serum glucose, data mentioned that carbon tetrachloride-treated rats exhibited a significant increase in the levels of serum glucose (146.71mg/dl) as compared to normal control (79.08mg/dl) and extract of *P. major* herbs-treated rats (68.27mg/dl). The application of *P. major* extract groups before CCl4 injection exhibited significant reductions in the levels of serum glucose (101.32mg/dl) as compared to *P. major* extract groups either with (131.27mg/dl) or after (135.91mg/dl) CCl4 injection.

The obtained results are in agreement with those reported by Atta *et al.* (2006) and Usoh *et al.* (2015), they observed the levels of serum glucose were increased with CCl4 treated.

On the other hand, Noor *et al.* (2000) found that 600 mg/kg dose of *P. major* water extract had a significant effect in reducing blood glucose level in diabetic rats. Also, Palmeiro *et al.* (2003) found that aqueous extract of *P. australis* leaves at 850 mg/kg decreased glucose serum levels in relation to control. Similarly, Ahmed *et al.* (2010) found that the blood glucose levels of treated groups of rats showed significant reduction after 7 weeks of treatment with *P. psyllium*. In addition, Shahat *et al.* (2012) showed the Serum glucose level showed significant increase in obese rats as compared with that in lean control rats. In contrast, significant decrease in serum glucose level was demonstrated in obese rats treated with *Plantago* seeds as compared with that in obese control rats.

Lipid profile

With respect to lipid profile, it is unveiled the groups of rats treated with *Plantago* extracts had a significant decrease in the levels of total cholesterol, triglyceride and low density lipoprotein cholesterol (LDL) with a significant increase in the level of in high density lipoprotein cholesterol (HDL) (149.08, 62.55, 76.69 and 52.25mg/dl respectively) as compared to CCl4 treated groups (178.19, 161.38, 91.64 and 33.64mg/dl respectively) and normal control (155.64, 153.87, 87.14 and 43.24 mg/dl respectively). The application of *P. major* extract groups before CCl4 injection exhibited significant reductions in these parameters

except HDL (158.28, 86.65, 82.37 and 47.80mg/dl respectively) as compared to *P. major* extract groups either with or after CCl4 injection.

The obtained results are in harmony with those reported by Atta *et al.* (2006) who found that oral administration of the methanolic extract (1000 mg/kg) of *P. major* seeds significantly decreased the levels of total cholesterol and triglyceride as compared to CCl4 treated rats and normal control. Also, Ahmed *et al.* (2010) found that cholesterol levels of treated groups of rats showed significant reduction after 7 weeks of treatment with *P. psyllium*.

TABLE 5. Effect of oral administration of *P. major* extract to rats injected with CCl4 on serum levels of glucose and lipid profile.

Treatment	Glucose (mg/dl)	Lipid profile			
		Chl (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Negative control (non-treated)	79.08	155.64	153.87	87.14	43.24
CCl4(0.8ml/Kg.b.wt)	146.71	178.19	161.38	91.64	33.64
P. major extract (100mg/Kg.b.wt)	63.47	149.08	62.55	76.69	52.25
P. major extract before CCl4	101.32	158.28	86.65	82.37	47.80
P. major extract with CCl4	131.27	161.39	102.96	84.29	40.55
P. major extract after CCl4	135.91	165.87	121.71	87.08	35.20
New LSD at 0.05 =	14.31	3.08	6.81	1.87	7.03

Liver functions

Our results showed that CCl_4 markedly increased serum aminotransferases (AST and ALT), alkaline phosphatase (ALPh), total protein, albumin (ALB) and total bilirubin activities as indicators of hepatic injury since elevated levels of these hepatocellular enzymes are signs of CCl_4-induced tissue damage which it record (44.32 U/L, 70.12 U/L, 312.59 U/L, 7.59 g/dl, 4.93 g/dl, and 0.92mg/dl respectively) as compared to normal control (13.43 U/L, 38.33 U/L, 147.61 U/L, 7.21 g/dl, 4.54 g/dl, and 0.53mg/dl respectively) and *Plantago* extracts treated groups (11.39 U/L, 33.09 U/L, 141.23 U/L, 7.16 g/dl, 3.51 g/dl and 0.50mg/dl respectively). Moreover, pretreatment with *P. major* methanolic extract significantly decreased the CCl_4-induced elevation of serum aminotransferases (ALT, AST) and ALPh activities and levels of serum total bilirubin, albumin and total bilirubin which may shows its action in preventing the acute tissue damage which it record (17.18 U/L, 40.92 U/L, 179.34 U/L, 7.25 g/dl, 3.99 g/dl and 0.55mg/dl respectively) as compared to *P. major* extract groups either with or after CCl4 injection. The potential mechanism of this effect is by enhancing the activities of innate antioxidants.

The obtained results are in agreement with those reported by Atta *et al.* (2006) who found that *P. major* is hepatoprotective activity against CCl4 toxicity. However, oral administration of the methanolic extract (1000 mg/kg) of *P. major* seeds significantly attenuated the CCl4-induced hepatotoxicity. Pretreatment with *P. major* methanolic extract significantly decreased the CCl4-induced elevation of serum aminotransferases (ALT, AST) and GGT activities. The levels of total bilirubin and albumin were also decreased. Also, Türel *et al.* (2009) showed that *P. major* extract (25 mg/kg) reduced the serum alanine amino transferase (ALT) and aspartate amino transferase (AST) levels compared to the treated rat of CCl4 induced hepatotoxicity. In addition, Usoh *et al.* (2015), showed that CCl4 treatment groups exhibiting significant decrease in liver functions including a significant increase in serum concentration of AST, ALT and ALPh compared to untreated rats.

TABLE 6. Effect of oral administration of *P. major* extract to rats injected with CCl4 on liver function.

Treatment (mg/Kg.b.wt)	AST (U/L)	ALT (U/L)	ALPh (U/L)	Total protein (g/dl)	(ALB) (g/dl)	Total bilirubin (mg/dl)
Negative control (non-treated)	13.43	38.33	47.61	7.21	4.54	0.53
CCl4 (0.8ml/Kg.b.wt)	44.32	70.12	212.59	7.59	4.93	0.92
P. major extract (100mg / Kg. b.wt)	11.39	33.09	41.23	7.16	3.51	0.50
P. major extract before CCl4	17.18	40.92	79.34	7.25	3.99	0.55
P. major extract with CCl4	28.39	57.41	107.98	7.33	4.38	0.61
P. major extract after CCl4	32.29	62.87	164.31	7.46	4.76	0.72
New LSD at 0.05 =	2.01	5.13	6.34	0.04	0.37	0.02

Kidney function

With respect to Kidney function, it is unveiled significant increases in the levels of serum urea, uric acid and creatinine in the groups of rats treated with CCl4 alone (91.74, 7.29 and 0.193mg/dl respectively) as compared to normal control (49.36, 4.94 and 0.168mg/dl respectively) and *Plantago* extracts treated groups (44.67, 4.63 and 0.159mg/dl respectively). The application of *P. major* extract groups before CCl4 injection exhibited significant reductions in these parameters (59.05, 5.52 and 0.168mg/dl respectively) as compared to *P. major* extract groups either with or after CCl4 injection.

The present study also revealed that the administration of CCl4 caused marked impairment in renal function alongside with significant oxidative stress in the kidney. Serum urea, creatinine, and uric acid concentrations were significantly higher in CCl4-treated rats. Urea is the main end product of protein

catabolism. Serum creatinine elevation was caused by CCl4 due to altered kidney function. It is one of the waste products of the body which is passed into blood stream to be removed by kidney. *P. major* extract significantly decreased the elevated levels of serum urea, creatinine, and uric acid, which indicates that the *p. major* extract possibly protects kidney tissue against oxidative damages induced by CCl4 and indicates maintenance of renal function.

The obtained results are in the same line with those reported by Adewole *et al.* (2007), Al-Yahya *et al.* (2013) and Usoh *et al.* (2015), they showed that CCl4 treatment groups exhibiting significant decrease in kidney functions including a significant increase in serum concentration of urea, uric acid and creatinine compared to untreated rats.

Moreover, Sierra *et al.* (2002) found that *P. psyllium* reduced uric acid (10%, significant difference). Also, Hong *et al.* (2011) found that *P. asiatica* L. extract pre-treated group showed decreases in the levels of serum urea and serum creatinine. Similarly, Mao-ye and Li-guo (2011) found that *P. major* L. seeds extract at 120mg/kg body weight decrease serum urea in the mice blood.

Generally, the protective effect of plant extracts against CCl4 may be attributed to the presence of flavonoids, tannins, (Gilani and Janbaz, 1995), triterpenoids and steroids among the plant constituents that possess hepatoprotective effect (Gupta *et al.*, 2004). Flavonoids are known to be antioxidants, free radical scavengers and antilipoperoxidants leading to hepatoprotection (Al-Qarawi *et al.*, 2004). The active compounds of *P. major* for the observed effects have not been identified in the present study. Many compounds known to be beneficial against CCl4-mediated liver injury exert their protective action by toxin-mediated lipid peroxidation either via decreased production of CCl4 derived free radicals or through the antioxidant activity of the protective agents themselves (Gilani and Janbaz, 1995).

Cytogenetic studies

Abnormalities in bone marrow (somatic) and sperm (germ) cells

Tables 8 and 9 showed the number and mean percentage of abnormalities induced in control and treated animals. The percentage of aberrant cells in animals treated with CCl4 induced a high percentage of abnormalities ($p<0.01$) in somatic and germ cells. While, the animal groups treated with ethanolic extracts of *P. major* was statistically non-significant in comparing to the control group. The crud extracts of *P. major* reduced the number of abnormalities when administered to CCl4 in all treatment. This reduction of abnormalities excluding gaps in bone marrow cells reached to 40%, 31% and 22% respectively (Table 8). While, for sperm abnormalities inhibition it reached to 66% , 45% and 31% before, with and after treatment with *P. major* respectively(Table 9). Our results showed that the crude extract of *P. major* is more effect when administered before and with CCl4 than that when treated after CCl4. Fig. 1 and 2 showing the different abnormalities in somatic and germ cells respectively.

TABLE 7. Effect of oral administration of plant extracts to rats injected with CCl4 on kidney function.

Treatment	Serum urea (mg/dl)	Serum uric acid (mg/dl)	Serum creatinine (mg/dl)
Negative control (non-treated)	49.36	4.94	0.168
CCl4(0.8ml/Kg.b.wt)	91.74	7.29	0.193
P. major extract (100mg/Kg.b.wt)	44.67	4.63	0.159
P. major extract before CCl4	59.05	5.52	0.168
P. major extract with CCl4	66.81	5.87	0.176
P. major extract after CCl4	72.41	6.29	0.187
New LSD at 0.05 =	4.61	0.27	0.007

TABLE 8. Number and mean percentage of chromosomal aberrations induced after treatment with CCl4 and CCl4 with ethanolic crud extract from *P. major* in rat bone marrow cells.

Treatments (mg/kg b.wt)	Total Abnormal Metaphases			No. of different types of metaphases					Inhibitory index Excluding Gaps
	No.	Mean(%) ± SE		G.	Frag. and/or Br.	Del.	M.A.	Polyp.	
		Including Gaps	Excluding Gaps						
I. control	20	4.00±0.48	2.20±0.40	9	7	4	0	0	-
II.CCl4 (0.8ml/kg)	103	20.60±0.52 [a]	16.60±0.65 [a]	20	52	8	17	6	-
III. P. major (100)	23	4.60±0.45	2.40±0.52	11	7	5	0	0	-
IV. *P. major* extract before CCl4	70	14.00±0.55[ab]	10.80±0.48[ab]	16	41	4	6	3	40
V. *P. major* extract with CCl4	81	16.20±0.50 [ab]	12.20±0.45 [ab]	20	44	8	4	5	31
VI. *P. major* extract after CCl4	88	17.60±0.48[ab]	13.40±0.50[ab]	19	50	7	8	4	22

Total number of examined metaphases 500 (5 animals/group), G.: Gap, Frag.: Fragments, Br.: Breaks, Del.: Deletions , M.A.: Multiple aberrations, Polyp: Polyploidy.
a: Significant compared to control ($p<0.01$), b: Significant compared to CCl4 treatment ($p<0.01$, t-test).

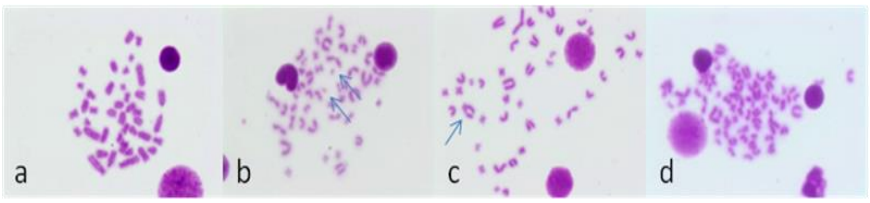

Fig.1. Chromosomal aberrations in rat bone marrow cells after treatment with CCL4 with ethanol crud extract of *P. major* showing, a) Normal, b) Fragments, c) Break and d) Polyploidy.

TABLE 9. Number and mean percentage of sperm abnormalities induced in rat after treatment with CCl4 and with ethanolic crud extract from *P. major*.

Treatments Mg/kg b.wt.	Abnormal sperms		No. of different types of sperm head abnormalities					Inhibitory index
	No.	Mean % ± SE	Straight	Banana shape	Amorphous	Without hook	Coiled tail	
I. Control	113	2.26±0.50	31	4	61	10	7	-
II.CCl4 (0.8ml/kg)	536	10. 72±0.56 [a]	75	56	206	146	53	-
III. *P. major* (100)	117	2. 34±0.48	28	7	65	12	5	-
IV. *P. major* extract before CCl4	257	5.14±0.72 [ab]	67	31	113	32	14	66
V. *P. major* extract with CCl4	351	7.02±0.44 [ab]	85	35	151	54	26	45
VI. *P. major* extract after CCl4	405	8.10±0.58 [ab]	101	47	148	78	31	31

NO of examined sperms 5000 (1000 /animal, 5 animals/group)
a: Significant compared to vehicle control ($p<0.01$), b: Significant compared to CCl4 treatment ($p<0.01$, t-test).

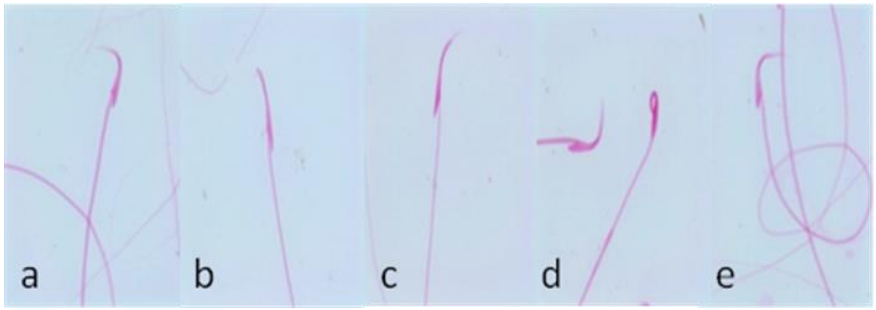

Fig.2. Sperm abnormalities in rat after treatment with CCl4 and with ethanol crud extract of *P. major* showing, a) Normal, b) Straight and without hook, c) Banana, d) Amorphous and e) Coiled tail.

Genotoxic activities of CCl4 have been reported in various assays. As for clastogenic effect of CCl4 the micronucleus assay *in vivo* was widely employed (Suzuki *et al.,* 1997 and Crebeli *et al.,* 1999). CCl4 was confirmed to cause cancer in rodents after prolonged exposure to CCl4 (Westbrook- Collins *et al.,* 1990). Positive clastogenic or aneugenic results after treatment with of CCl4 were found in human metabolically competent cell lines, MCL-5 CYP which expresses cDNA encoding the human CYP1A1, and h2E1 cell lines containing cDNA for CYP2E1 (Doherty *et al.,* 1996). Carbon tetrachloride in combination with chlordecone was genotoxic using the *in vivo – in vitro* animal model and a battery of biochemical assays to measure the DNA repair in the rat hepatocytes (Ikegwuonu and Mehendale, 1991). Because of long-lived tissue contacting different organs and organic systems, and that they have a potential to accumulate DNA lesions (Carrano and Natarajan, 1988) the peripheral lymphocytes are considered to be suitable for *in vivo* assays (Rojas *et al.,* 1992). A significant increase of SCE ($p < 0.05$, $p < 0.001$) in sheep peripheral lymphocytes after CCl4 treatment in both assays i.e. with and without protective agent administration (Dianovsk and Ivikova, 2001). These previous studies are in the same line with our study which we found that administration CCl4 at 0.8ml/kg b.w. to rat *in vivo* induced a significant increase on chromosomal aberrations and sperm abnormalities compared to negative control.

Antigenotoxicity of ethanolic extracts of *P. major* was established in bone marrow and sperm cells in CCl4 treated rat. Ethanolic extracts of *P. major* at a dose of 100 mg/kg b.w. had no apparent genotoxic effect, as the proportion of aberrant cells was not significantly different from that of the negative control in both somatic and germ cells, whereas conversely, it displayed significant antigenotoxic activity against CCl4 induced mutagenesis in bone marrow and sperm cells. CCl4, an extensively studied hepatotoxin is converted into its metabolites such as CCl3 radicals which are involved in the liver pathogenesis including cirrhosis, genotoxicity of hepatic tissue and hepatic carcinoma (Khan *et al.,* 2012). El-Souda *et al.* (2014) demonstrated that *P. albicans*

phytoconstituents play a protective role against genotoxicity of the drug cyclophosphamide in mouse bone marrow cells.

Methanolic and ethanolic extracts of *P. major* have potential to be used as an alternative or adjunct treatment to reduce inflammation-mediated cell injury following APAP toxicity (Hussan *et al.,* 2015).

P. major is one of the most studied plants. Most of its components and the pharmacological action of some of them are well known (Velasco-Lezama *et al.,* 2006). It is possible that acids known to be contained in the plant (caffeic, ferulic, chlorogenic, ursolic and oleanic acids), with proven antitumor activity *in vitro* (Liu, 1995). *P. major* also contains flavonoids namely, luteoline, apigenin, hispidulin, baicalein, etc., known for their capability of inducing carcinoma cell death (Matsuzaki *et al.,* 1996). Galvez *et al.* (2003) reported that *Plantago* extracts have growth inhibitory and cytotoxic effects of breast adenocarcinoma and melanoma cell lines and these preliminary results could be justified by the cytotoxic activity of the flavone, luteolin-7-*O*-β-glucoside, the major flavonoid in *Plantago* species.

Our results showed that *P. major* ethanolic extracts have the ability to reduce DNA damage induce in somatic and germ cells before, with and after treatment with CCl4. These results are in agreement with Atta *et al.* (2006) and Turel *et al.* (2009) they reported that *P. major* has a hepatoprotective effect when administered prior to CCl4 exposure. While, Hussan *et al.* (2015) reported that administration of *P. major* after rat liver injury exposed to acetaminophen reduced this damage.

We speculate that *P. major* mediate their chemopreventive effects against CCl4 either via decreased production of CCl4 derived free radicals or through the antioxidant activity of the protective agents themselves (Oto *et al.,* 2011, Mello *et al.,* 2015 and Farag *et al* ., 2015).

In conclusion, ethanolic extracts of *P. major* proved to be nontoxic and to exhibit hepatoprotective and antigenotoxic potential.

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تقييم التأثير الوقائي من المستخلص الإيثانولى لنبات لسان الحمل ضد تأثير رابع كلوريد الكربون المسببة لحدوث السمية الوراثية والاضطراب الكيميائي الحيوي في الفئران

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لقد أجريت هذه الدراسة خلال مواسم ٢٠١٠-٢٠١٤، على الأعشاب الطازجة من نبات لسان الحمل، والتي تم جمعها من مشتل خاص بمدينة القناطر الخيرية، التابعة لمحافظة القليوبية بجمهورية مصر العربية مصر لدراسة التأثير الوقائي من المستخلص الإيثانولى لنبات لسان الحمل ضد تأثير رابع كلوريد الكربون المسببة للتوتر الناجم عن الأكسدة في الأنسجة المختلفة في الفئران.

وقد أظهرت النتائج أن الفئران المعاملة برابع كلوريد الكربون زيادة كبيرة في مستويات الجلوكوز في مصل الدم والكوليسترول، الدهون الثلاثية و البروتين الدهني المنخفض الكثافة الكولسترول (LDL) مع انخفاض كبير في مستوى في البروتين الدهني العالي الكثافة الكولسترول (HDL) من خلال تحليل صور الدهون فى الدم كما أدت أيضا إلى خلل فى وظائف الكبد بما في ذلك زيادة إنزيمات الأمينوترانسفيريز (AST and ALT)، إنزيم الفوسفاتيز القلوية (ALPH) ، البروتين الكلي، الألبيومين (ALB) ونشاط البيليروبين، بالاضافة إلى ذلك وظائف الكلى متمثلة في زيادة مستويات اليوريا في الدم، وحمض اليوريك ونشاط الكرياتينين، وقد ادى المعاملة بالمستخلص الإيثانولى لنبات لسان الحمل بالجرعة ١٠٠ مليجرام/كجم من وزن الفأر قبل ، ومع ، وبعد معاملة الفئران برابع كلوريد الكربون الى تحسين كل هذه التغييرات الكيميائية الحيوية بدرجة كبيرة .

ومن جهة أخرى، قد أحدثت المعاملة بالمستخلص الإيثانولى لنبات لسان الحمل تأثير معنوى فى تقليل التأثير الضار الناجم من استخدام رابع كلوريد الكربون على الحمض النووي في نخاع العظام والحيوانات المنوية فى الفئران .

من هذه النتائج التي توصلنا إليها يمكننا القول أن المستخلص الإيثانولى لنبات لسان الحمل له تأثير وقائي مهم ضد السمية فى الكبد والطفرات فى الحمض النووي الناجمة عن الاصابة بمركب رابع كلوريد الكربون في الفئران ، وهذا التحسن يمكن ارجاعه الى خصائص النبات المضادة للأكسدة.

الكلمات الدالة: لسان الحمل، رابع كلوريد الكربون، سمية أنسجة الكبد، شذوذ الكروموسومات والتحليل الكيميائي الحيوي.